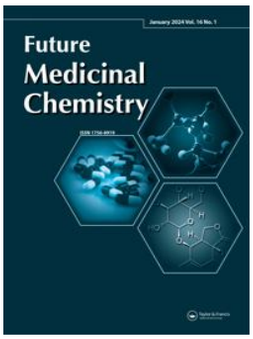

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Review

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Future Medicinal Chemistry

Nanomedicine in the treatment of diabetic nephropathy

Nimeet Desai[‡,1], HariPriya Koppisetti[‡,1], Shreya Pande[‡,1], Havish Shukla[‡,1], Bhagwat Sirsat[1], Aayushi S Ditani[1], Pragyan P Mallick[1], Umesh Kathar[1], Kiran Kalia[1] & Rakesh K Tekade*[,1]

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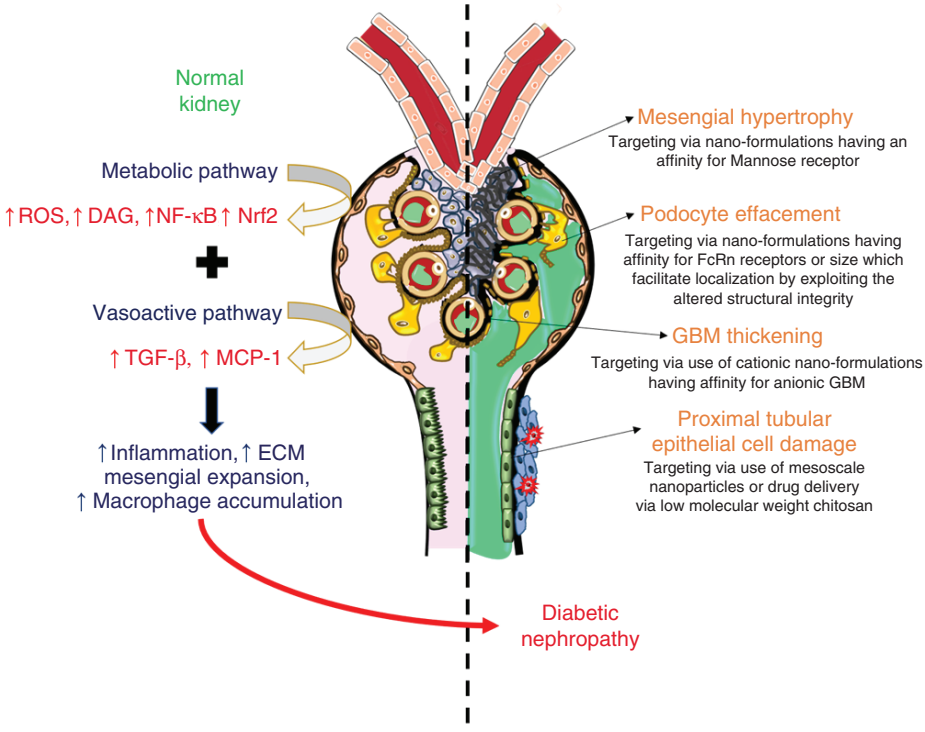
Globally, diabetic nephropathy (DN) is the foremost cause of end-stage renal disease. With the incidence of diabetes increasing day by day, DN's occurrence is expected to surge to pandemic proportions. Current available therapeutic interventions associated with DN emphasize blood pressure, glycemia and lipid control while ignoring DN's progression mechanism at a molecular level. This review sheds light on the molecular insights involved in DN to help understand the initiation and progression pattern. Further, we summarize novel strategies with reported applications in developing a nanomedicine-based platform for DN-targeted drug delivery to improve drug efficacy and safety.

Graphical abstract:


Normal kidney
Metabolic pathway
↑ROS, ↑DAG, ↑NF-κB ↑ Nrf2
Vasoactive pathway
↑ TGF-β, ↑ MCP-1
↑Inflammation, ↑ ECM mesengial expansion, ↑ Macrophage accumulation
Diabetic nephropathy
Mesengial hypertrophy
Targeting via nano-formulations having an affinity for Mannose receptor
Podocyte effacement
Targeting via nano-formulations having affinity for FcRn receptors or size which facilitate localization by exploiting the altered structural integrity
GBM thickening
Targeting via use of cationic nano-formulations having affinity for anionic GBM
Proximal tubular epithelial cell damage
Targeting via use of mesoscale nanoparticles or drug delivery via low molecular weight chitosan

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Keywords: diabetic nephropathy • molecular mechanics • nanomedicine • targeted drug delivery

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Background

Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder that affects the human body's process of converting food into energy. This condition is recognized by the presence of hyperglycemia in a fed or fasted state [1]. According to data published by the International DM Federation, 463 million people (9.3% of the global adult population) are estimated to be living with DM, and the number of people with DM will rise to 578 million by 2030 [2].

Various pathogenic processes play an essential role in the development of DM. These processes include the deficiency in insulin secretion due to β cells' altered functioning or the development of resistance to the insulin by body cells. This also results in the abnormal metabolism of fats, carbohydrates and proteins [3]. The fundamental risk factors for DM include obesity, physical inactivity, genetic polymorphism, cigarette smoking and pancreatic β cell dysfunction [4]. When the blood glucose level increases to more than 130 mg/dl, the risk of DM increases [5]. Based on the presence or absence of autoantibodies against pancreatic antigens and the age of the patient at diagnosis, DM is primarily classified into two types: Type 1 DM (also called insulin-dependent DM), caused by autoimmune β cell destruction which results in insulin deficiency, and Type 2 DM (also called non-insulin-dependent DM), wherein insulin secretion is eventually reduced due to the development of resistance by body cells toward insulin. Roughly 10% of patients with diabetes develop latent autoimmune diabetes in adults, which is a subgroup of DM characterized by the presence of glutamic acid decarboxylase antibodies. This condition is phenotypically indistinguishable from Type 2 diabetes at diagnosis but, with the passage of time, becomes increasingly similar to Type 1 diabetes [6].

The other types include drug-induced or chemical-induced DM (use of glucocorticoids), disease of the exocrine pancreas (cystic fibrosis, pancreatitis), monogenic diabetic syndrome (neonatal DM or maturity-onset DM of the young) and DM due to different syndromes (e.g., Down syndrome, Cushing syndrome) [7–9].

The elevated levels of glucose in DM eventually cause damage to many body organs, such as the heart (hypertension), eye (retinopathy), peripheral nervous system (neuropathy) and kidney (nephropathy) [10]. Hyperglycemia promotes macrovascular as well as microvascular complications. Macrovascular complications are associated with stroke and acute coronary syndrome, while microvascular complications are related to the microvessels responsible for regulating peripheral and renal blood pressure [11]. Alteration in renal blood pressure is one of the major causes of diabetic nephropathy (DN). DN is the leading cause of end-stage renal disease (ESRD), which is responsible for high mortality rates and disabilities in patients with DM [12,13]. Among the people living with DM, it is reported that approximately 50% are diagnosed with ESRD. Around 25% of patients with DM are predisposed to develop DN [14]. DN affects one in four women and one in five men with Type 2 diabetes.

Diabetic nephropathy

DN is a condition that occurs as a consequence of the interaction between metabolic and hemodynamic factors. The metabolic factors include glucose, advanced glycation end products (AGEs) and oxidative stress. These interactions between hemodynamic and metabolic factors cause the activation of intracellular secondary messengers such as PKC, nuclear factors (NF-κB), MAPK and various growth factors (TGF-β, VEGF), which causes induction and progression of diabetic nephropathy [15,16]. DN is more common in patients with Type 1 DM than in those with Type 2 DM [12]. In 15–40% of patients with Type 1 DM, proteinuria is observed, while in patients having Type 2 DM, 5–20% cases show proteinuria [17]. Figure 1 depicts the structural change brought about by DN in normal kidney.

Microalbuminuria is the first clinical indication for nephropathy and is defined by the presence of albumin in the urine in quantities exceeding 30 mg/24 h or 20–200 μg/min in an overnight urine sample. Macroalbuminuria refers to the condition when urine albumin excretion exceeds 300 mg/24 h or 200 μg/min [18]. Patients with microalbuminuria are referred to as having 'incipient' nephropathy, and those with macroalbuminuria as having 'overt' nephropathy. Patients have elevated glucose levels in the blood, and low glycemic control produces structural changes in the kidney. These changes are characterized by the thickening of the glomerular basement membrane (GBM), podocyte loss, mesangial cell expansion, glomerular hypertrophy, glomerulosclerosis, tubular atrophy and tubulointerstitial fibrosis, as shown in Figure 1 [16,19]. Normal glomerular filtration rate (GFR) denotes the functioning of a healthy kidney, and any change in routine functioning of the kidney can be diagnosed by measuring GFR. Methods used in the determination of GFR are based on creatinine clearance and estimated GFR

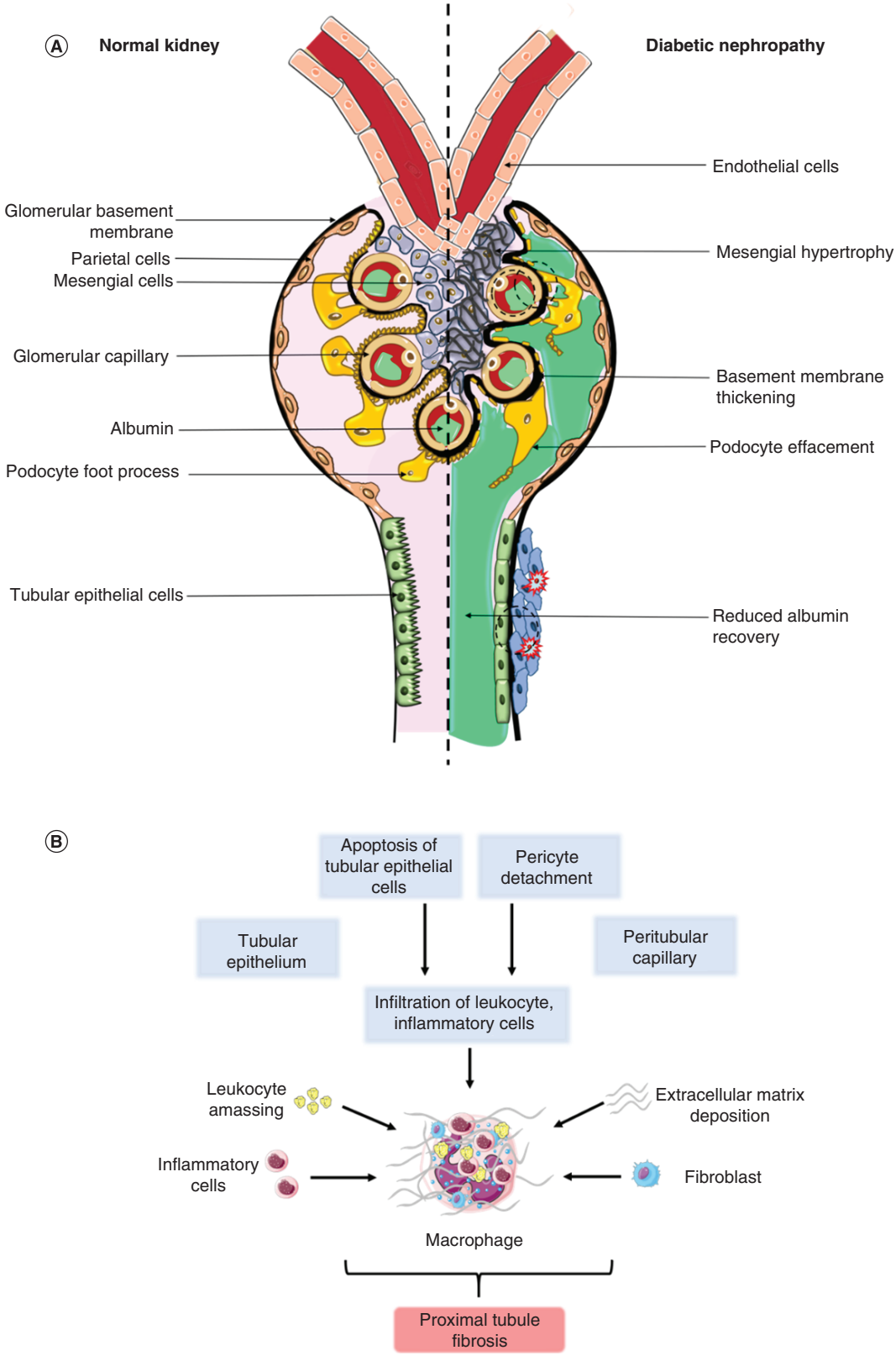

Figure 1. Depiction of the structural changes induced by diabetic nephropathy in normal kidney. The early-stage changes that occur in the functioning of the kidney during DN. **(A)** DN kidney is characterized by the expansion of mesangial cells, podocyte effacement, thickening of the glomerular basement membrane and reduced recovery of albumin. **(B)** The mechanism for proximal tubule fibrosis, which is a late-stage change. Renal oxidative stress enhances as an effect of albuminuria, which results in inflammation, tubular apoptosis and fibrosis.
DN: Diabetic nephropathy.

Table 1. Classification of diabetic nephropathy.

	Class/lesion	Description	Inclusion criteria	Ref.
Glomerular lesions	I	GBM thickening (EM) Mild (or nonspecific) changes (LM) An early indication of kidney damage often described as a prediabetic lesion Thickening occurs due to the accumulation of extracellular matrix components (collagen IV, laminin, fibronectin)	GBM >395 nm in females and >430 nm in males ≥9 years of age	[23–27]
	IIa (mild mesangial expansion) IIb (severe mesangial expansion)	Similar to diffuse diabetic glomerulosclerosis, mesangial cell expansion leads to glomerular capillary distortion; expansion is measured in fractional volume or volume density (Vv)	Mesangial expansion >25%	
	III (Kimmelstiel–Wilson lesion)	Referred to as nodular sclerosis; appear as focal, lobular Occurs in two steps: mesangiolysis and detachment of endothelial cells from GBM, oval to the round lesion of mesangium Often seen with mesangial lesions; can also be seen in hypertensive nephropathy	At least one nodular lesion	
	IV (advanced diabetic glomerulosclerosis)	Caused by excessive accumulation of extracellular matrix components (e.g., collagen I, III, IV and fibronectin)	Glomerular sclerosis >50%	
Tubulointerstitial lesions	IFTA	Shows tubular basement membrane thickening, followed by glomerular changes, which leads to ESRD. The score is given based on the total involved area of tubules and interstitium (0: no IFTA; 1: <25% IFTA; 2: 25–50% IFTA, 3: >50% IFTA)	Presence of mononuclear cells in the interstitium	[28]
	Interstitial inflammation	The score is given based on infiltration concerning IFTA (0: no interstitial infiltrates; 1: infiltrates only around atrophic tubules; 2: infiltrates in areas other than atrophic tubule)	T lymphocyte and macrophage infiltration	
Vascular lesions	Arteriolar hyalinosis (efferent arteriole)	Distinguishes DN from hypertensive nephropathy The score is given based on biopsy results (0: no arteriolar hyalinosis; 1: one arteriole with hyalinosis; 2: more than one arteriole hyalinosis)	-	[22,29]
Other	Insudative lesion	Classified based on the location of lesions: Bowman's capsule (capsular drop lesions) Glomerular capillary (fibrin cap lesions) Glomerulotubular junction (tip lesions)	Intramural accumulation of lipids and plasma proteins in renal arterioles	[26,29]

DN: Diabetic nephropathy; EM: Electron microscopy; ESRD: End-stage renal disease; GBM: Glomerular basement membrane; IFTA: Interstitial fibrosis and tubular atrophy; LM: Light microscopy.

(eGFR). In DN, there is an initial increase in albumin excretion rate, which then progresses to microalbuminuria and reduced GFR (eGFR is less than 60 ml/min) [20,21].

Classification of DN

The classification of DN is based on renal biopsy observations, in which renal tissue is stained using periodic acid–Schiff, Masson trichrome, occasional acid methenamine silver or hematoxylin and eosin. These stained renal tissues are observed using either electron microscopy (EM) or light microscopy (LM). The classification is given in Table 1 [22].

Diagnosis of DN

The presence of albumin in the urine is the first indication of diabetic nephropathy; albumin is the most definitive compound detected along with creatinine in renal damage [30,31]. However, the condition of micro- or macro-albuminuria may return to normal levels of albumin, and hence other biomarkers for accurate diagnosis of DN were established. These biomarkers are the indicators that are helpful in the detection of the pathological condition, and some of them are listed in Table 2 [32]. miRNA can also be used for the detection of DN as it is stable and easily detected in human urine [33]. In early-stage DN, urinary exosomal miRNA is detected. Other miRNAs used as a biomarker include miR-192, miR-21, miR-770 and miR-126 [33,34].

Here we review the various mechanisms behind the progression of DN in a constructive way to explain every possible factor that leads to DN; we also discuss targeted treatment modalities. Diabetes leads to hyperglycemia, which is associated with various factors causing hypertension and ultimately leads to dysfunction of the renal system due to altered pressure. Thus, DN is broadly defined in terms of renal impairment due to uncontrolled glycemic levels in the body, which lead to kidney failure. The failure in kidney functions leads to conditions like anemia, electrolyte imbalance, proteinuria and heart arrhythmia.

Table 2. Biomarkers used in the diagnosis of diabetic nephropathy.

Marker	Name	Description	Biomarker function	Ref.
Protein markers	Albumin	Albumin is considered a gold standard of biomarkers Indicates substantial organ damage	Diagnosis (upregulation of serum protein fragments)	[35–37]
	Type IV collagen	The decline in eGFR is seen with the presence of type IV collagen in the urine An early indicator of renal damage	Diagnosis (downregulation of collagen fragments in urine)	
	Adiponectin	Adipocyte-secreted cytokine Decreased plasma levels are associated with obesity, insulin resistance	Diagnosis (elevated levels in urine; acts as a novel urinary biomarker for normoalbuminuric DM patients)	[38]
	TNFR1/2, FGF23	TNFR indicates an early loss in renal function FGF23 level >70 pg/ml indicates a higher risk of DN (dialysis is needed)	Prognosis (higher urinary excretion associated with microalbuminuria; levels decrease with treatment)	[39]
Podocyte biomarkers	Nephrin, podocin, synaptopodin, podocalyxin	Expression of slit diaphragm proteins (nephrin, podocin, synaptopodin) is reported to be downregulated	Diagnosis (higher podocalyxin levels associated with microalbuminuria; early marker of podocyte injury)	[40,41]
Tubulointerstitial biomarkers	Urinary NGAL	Elevated levels are seen in normoalbuminuric patients Correlated with eGFR and albuminuria	Prognosis (urinary levels are increased significantly)	[42]
	Urinary NAG		Early diagnosis (earlier elevated urinary excretion than albumin)	
	Urinary H-FABP		Diagnosis (elevated levels in urine indicate distal tubular damage)	
	Urinary KIM-1	Not associated with eGFR and albuminuria Decreased level on treatment with ARB	Response to treatment (decreased value indicates healing of lesions)	

ARB: Angiotensin II receptor blocker; DM: Diabetes mellitus; DN: Diabetic nephropathy; eGFR: Estimated glomerular filtration rate.

Molecular mechanisms involved in DN

DN starts with the dysfunction of the glomerulus, composed of the afferent artery, Bowman's capsule and efferent artery. The significant conditions that lead to the progression of DN are: hemodynamic changes in afferent and efferent artery constriction; glomerulosclerosis; and mesangium expansion. In DM, the upregulation of the renin–angiotensin–aldosterone system (RAAS) is observed, which constricts the arteries (mainly the efferent artery). Due to vasoconstriction, the blood pressure increases in the glomeruli, creating an intraglomerular pressure. AGEs and PKC increase vascular permeability, damaging the basal membrane [43]. Due to intraglomerular pressure and thickening of the basal membrane, more blood is passed into the mesangium, causing it to expand and decrease in surface area for glomerular filtration. The podocytes of the vascular epithelium proliferate on the expansion of the mesangium, leading to the leakage of blood into Bowman's capsule and causing macroalbuminuria due to the disruption of podocytes around the glomerular basement membrane. Podocytes, which play a role in the synthesis of elements for the GBM, also help in counteracting the hydrostatic pressure in capillaries. They are negatively charged, due to which the passage of negatively charged molecules like albumin across the GBM is limited [44]. In DN the expression of integrin protein α3β1, responsible for adherence of podocytes around the GBM, is reduced, resulting in a leaky membrane and thickening of the GBM which allows the accumulation of proteins in the mesangia. Lack of podocytes also leads to an increase in hydrostatic pressure in capillaries, as well as developing an oncotic pressure. All these conditions together correspond to a decrease in GFR [45]. To simplify the DN's molecular mechanisms, they are broadly classified into vasoactive and metabolic pathways. Each pathway, with its factors leading to intraglomerular pressure, is discussed in detail below.

Vasoactive pathways

RAAS pathway

RAAS is considered the primary cause of intraglomerular pressure in DN. The kidney's juxtaglomerular cells produce a proteolytic enzyme, renin, which converts angiotensinogen to angiotensin I (Ang I). Ang I is converted to angiotensin II (Ang II) in a process mediated by angiotensin-converting enzyme (ACE). Ang II is a vasoconstrictive agent that acts directly on vascular smooth muscles, causing them to constrict and increase blood pressure. This also increases the generation of aldosterone by the adrenal cortex and promotes sodium reabsorption [46].

Ang II binds with the receptors AT_1R and AT_2R, causing vasoconstriction via AT_1R and vasodilation via AT_2R. In the condition of hypertension associated with DM, the vasoconstriction of the efferent artery is greater than in

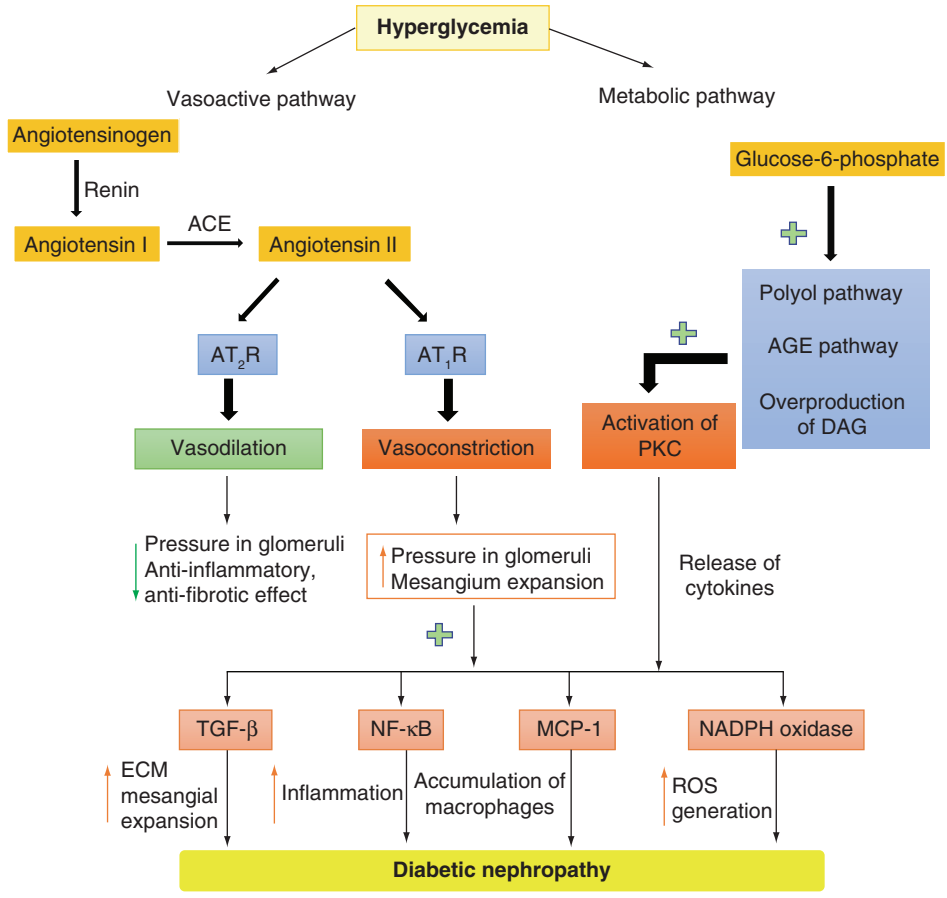

Figure 2. Diagrammatic representation of pathways responsible for diabetic nephropathy.
ACE: Angiotensinogen converting enzyme; AGE: Advanced glycated end product; DAG: Diacylglycerol; ECM: Extracellular matrix; ROS: Reactive oxygen species.

the afferent artery. This causes the accumulation of more blood and increases the pressure in Bowman's capsule, leading to the expansion of the mesangium and hypertrophy of tubular epithelial cells, which ultimately leads to glomerular hypertension [47]. As an effect of the RAAS pathway, inflammation is seen, which leads to the release of various cytokines (TGF-β, MCP-1, NF-κB), which affects the kidney (Figure 2).

To block the RAAS, ACE inhibitors and angiotensin II receptor blockers (ARBs) can be given. ACE inhibitors decrease the synthesis of Ang II, thereby reducing vasoconstriction and glomerular hypertension, whereas ARBs block the AT_1R, thus making Ang II bind with AT_2R, which induces vasodilation and reduces the intraglomerular pressure and expansion of the mesangium [48]. The chances of renin elevation increases if ACE inhibitors or ARBs are employed to treat hypertension associated with DM. To anticipate that effect, a direct renin-blocking agent, aliskiren, is prescribed. This direct renin inhibitor has been shown to be efficient in treating DN along with ACE inhibitors and ARBs [49].

Hypertrophy of the mesangium & tubular epithelial cells

Due to the increased levels of Ang II and vasoconstriction, intraglomerular blood pressure increases, leading to inflammation and expansion of the mesangium and tubular epithelial cells. These cells release cytokines such as TGF-β, MCP-1, VEGF and NF-κB as part of the inflammatory response [48].

TGF-β

TGF-β is a prosclerotic cytokine that belongs to the cytokine family that forms complexes with type 1 and type 2 serine/threonine kinase receptors, which regulate gene expression associated with growth arrest, immune system control, cell differentiation, angiogenesis and epithelial–mesenchymal transition by altering nucleosome

structure [50]. Due to the inflammatory response of mesangial and tubular epithelial cells, production of TGF-β is upregulated, inducing the secretion of PDGF-A, which promotes epithelial–mesenchymal transition. That leads to the expression of the α-smooth actin that characterizes interstitial myofibroblasts, which are components of the tubulointerstitial extracellular matrix [51]. The deposition of extracellular matrix increases vascular permeability and makes the glomeruli leaky, leading to proteinuria and intraglomerular hypertension. TGF-β is not only induced due to inflammation of mesangium, but is also upregulated due to increased glucose levels in the blood that result in the activation of PKC and AGE. Hence targeting TGF-β can minimize the inflammatory response and reduce the damage to mesangial cells and tubular epithelial cells [52].

MCP-1

MCP-1 is a chemokine, also referred to as a monocyte chemotactic and activating factor. Mesangial cells and tubular epithelial cells of glomeruli produce MCP-1 in response to upregulation of the *MCP-1* gene, triggered due to protein overload and increased glucose levels [53]. MCP-1 recruits activated macrophages/monocytes into the renal tubulointerstitium, leading to leakage of the glomerular endothelial membrane, which causes renal damage [54]. Macrophages play a vital role in immune responses and have the ability to switch their phenotype depending on the microenvironment. M1 and M2 are the two main phenotypes of macrophages found in immune responses [55]. M1 is a classically activated proinflammatory macrophage, whereas M2 has an anti-inflammatory effect. M1 initiates the process by inducing inflammatory mediators, which are followed up by M2, which show a response to overcome inflammation. M1 is promoted by IL-6 and TNF-α, the proinflammatory mediators, and M2 is promoted by IL-4, IL-13 and M-CSF. In conditions like DN, M1 and M2 coexist, producing continuous inflammation and fibrosis. However, the M1 phenotype is of concern because M1 macrophages are also stimulated as a response from the NF-κB pathway [56].

According to a study conducted by Chen *et al.* on recombinant rats, Ang II directly induces the expression of *MCP-1* in vascular smooth muscle [57]. *MCP-1* is also activated or induced by AGEs and reactive oxygen species (ROS), as discussed in a later section. To downregulate *MCP-1* expression, Ang II levels can be reduced by using ACE inhibitors [58].

Role of calcium & potassium channels

Afferent arteriolar dilatation is seen in the early stages of DM, associated with the diminishing response to vasoconstriction. Ca^{2+} and K^{+} channels play a significant part in maintaining the tone of vascular smooth muscles. Vascular afferent arterioles depend on Ca^{2+} influx through voltage-dependent calcium channels (VDC) [59]. These channels contribute to vasoconstriction, which in turn contributes to hypertension. Alteration in the intracellular Ca^{2+} level directly activates calcium-binding proteins, which are a response to damage-associated molecular patterns (DAMPs). DAMPs are endogenous danger molecules that are released on cellular stress or damaged tissues, in turn activating the innate immune system by interacting with pattern recognition receptors [60]. DAMPs contribute to host defense and promote pathological inflammatory responses. They activate various inflammatory proteins like S100, heat shock proteins, HMGB1 and fibronectin. [61]. S100 proteins are calcium-binding proteins released due to an increase in calcium in the cells, and are known to regulate several cell functions [62]. Also, as the response to DAMPs, HMGB1 is released into the extracellular space, which produces an inflammatory response by activating the NF-κB pathway [63].

In an experimental investigation of renal function in streptozotocin-treated mice by Carmines *et al.*, VDC agonist and K^{+}-induced depolarizations triggered the afferent arteriole response. Instead, depolarization-induced Ca^{2+} influx leads to increased intracellular Ca^{2+} levels. This suggested that hyperglycemia alters the function of afferent arterioles by dysregulation of vascular membrane potential by overexpression of K^{+} channels, leading to hyperpolarization and reducing the Ca^{2+} via VDC, causing vasodilation [64]. It was evident that dilatation of the afferent artery in the initial stages leads to more blood efflux into the glomeruli, which activates PKC and AGEs that lead to renal damage [43].

Endothelin receptor-mediated pathway

Endothelin is a group of cell-derived peptides produced by mesangial, glomerular epithelial, renal tubular and medullary collecting tubule cells. The endothelin system is a group of 21-amino acid peptides comprising ET-1, ET-2 and ET-3. ET-1 is mostly expressed in the kidney and mediates its action via G-protein-coupled receptors, ET_A and ET_B [65]. ET_A is located on the vasa recta and arcuate arteries in the glomeruli, whereas ET_B is present on

the endothelial and epithelial cells lining the renal tubule. The receptors function in opposition, one upregulating and the other downregulating endothelin functions.

In the conditions of renal impairment and DM, ET-1 is overexpressed due to hyperglycemia and insulin, leading to abnormalities in microvasculature on mesangial cells and podocytes [66]. ET-1 acting via ET_A in these conditions ultimately leads to glomerulosclerosis, inflammation, vasoconstriction, mesangial proliferation and podocyte injury, resulting in the activation of various transcription factors, growth factors and cytokines [67,68]. Endothelin-targeted therapeutic modalities can be adapted to inhibit the upregulation of associated factors. Atrosentan and avosentan are the current drugs targeting endothelin antagonism to downregulate its action.

Metabolic pathways

Generation of ROS

In hyperglycemia, the increased glucose levels in cells may lead to electron leakage from the cells, causing an oxidative stress condition due to the generation of ROS [69]. ROS play a crucial role in intracellular signaling, but their overproduction and accumulation creates a condition of oxidative stress that leads to the damage of cellular components (DNA and proteins) and may even lead to cell death. Overproduction of ROS in renal endothelium or underlying cells causes cellular damage and ruptures the basal membrane of glomeruli [70]. The activation of ROS generation also mediates various other pathways, like PKC, NF-κB, polyol and AGE formation (Figure 2).

PKC pathway

The increased glucose levels in hyperglycemia contribute to the formation of diacylglycerol (DAG) by glycolytic intermediate dihydroxyacetone phosphate on reduction with glucose-3-phosphate and various other metabolites of glycolysis [71]. This results in a stepwise formation of DAG. Apart from glycolysis, phospholipase D from lipoproteins and oxidative glycated products also lead to DAG formation [72].

An increase in DAG levels activates PKC, which is one of the factors responsible for the activation of TGF-β. The DAG–PKC pathway enhances Ang II to act via AT_2R and also increases vasodilatory prostaglandins. That results in vasodilation of the afferent artery, causing more blood to enter the glomeruli [73]. PKC is a group of serine/threonine kinases with 12 isoforms that bind to Ca^{2+} and DAG. Activation of PKC also results in the activation of various other factors (e.g., VEGF-α) to increase capillary permeability [74]. Thus it is clearly explained that binding of PKC with Ca^{2+} and through DAG synthesis leads to the formation of MAPK oxidases that induce TGF-β and result in proteinuria, mesangial expansion and ultimately glomerulosclerosis [75].

AGEs

AGEs are formed due to permanent changes in DNA, lipids or proteins caused by increased blood glucose levels, making glucose bind with several amino acids in a cross-linking complex [76]. This cross-linking complex is formed in an extracellular matrix that makes arteriolar walls and basement membrane stiff by activating AGE receptors [76,77]. Binding with the receptor mediates the overproduction of various transport factors (e.g., TGF-β, MCP-1 and VEGF-α) in mesangial cells and podocytes. Apart from these, it also induces oxidative stress in the mitochondria, leading to ROS generation [69]. All these factors associated with AGEs lead to disruption of cells in the epithelium and mesangium, leading to the porous basement membrane and leaky vasculature that result in proteinuria, glomerulosclerosis and finally renal damage [78].

Polyol pathway

The polyol pathway deals with the conversion of glucose to sorbitol using NADPH, mediated by the enzyme aldose reductase. In hyperglycemia, aldose reductase activity is increased due to an increased amount of glucose conversion [79]. This pathway results in certain conditions that lead to renal damage: the accumulation of sorbitol in the cell causes osmotic stress; aldose reductase consumes a larger amount of NADPH, leading to a lack of antioxidants in the cell and creating a condition of oxidative stress; and the previous factor leads to an increase in the $NADH/NAD^+$ ratio that activates the PKC pathway. The products formed by this pathway are more potent glycosylating agents than glucose, which leads to increased production of AGEs. Altogether, these factors contribute to the renal damage in DN [80]. One of the critical steps to target the polyol pathway is to inhibit the activity of aldose reductase. Tolrestat and epalrestat are drugs which have been proven to be potential aldose reductase inhibitors for use in DN [81].

Transcription factors NF-κB & NRF2

In the condition of hyperglycemia, upregulation of various transcription factors (e.g., NF-κB and NRF2) is seen. NF-κB is a p50–p65 heterodimer, which in complex with the inhibitory protein IκB exists in an inactive state in cytoplasm. IκB degrades upon action by IκB kinase, which is induced by high glucose phosphorylation [82]. The degraded IκB releases the NF-κB into the nucleus and triggers the genes. This NF-κB is released not only as a direct response to high glucose, but also as an inflammatory response by activation of the PKC pathway. This also binds to several promoter genes like *MCP1*, *TGFB1*, *CCL2* (encoding C-C motif chemokine 2) and *ICAM1* (encoding intercellular adhesion molecule 1). These genes have been identified as playing a role in the progression of DN [76,83,84]. NF-κB is upregulated due to excess ROS and AGEs; NRF2 is found mostly in the kidney, shows negative feedback control on NF-κB and promotes antioxidants [85]. Targeting the transcription factors is an emerging approach in the treatment of DN. Pyrrolidine dithiocarbamate and bardoxolone methyl have been used in a clinical trial for targeting DN as NF-κB inhibitors and NRF2 agonists [86].

Other minor pathways

Other minor pathways responsible for the progression of DN are the JAK-STAT pathway, autophagy, ASK-1, MAPK pathway, fibrosis, epigenetic factors (that contribute to DNA modification and posttranslational changes) and the use of statins (antihyperlipidemic drugs) [76].

Strategies for targeting DN

Treatment of nephropathic conditions demands that high concentrations of drugs reach the kidney, and this is generally associated with various adverse effects. In DN, when the drug is administered in high concentration, it does not ensure that the target cell will receive the required amount of drug because of impaired glomerular filtration and tubular secretion mechanisms. Kidney-targeted drug delivery helps to overcome this problem [87].

Commonly used strategies to deliver the drug at the desired site include prodrug development, using macromolecular carriers and others (nanoparticles, liposomes). Prodrugs are converted to the active drug form by the action of renal enzymes. In macromolecular carriers, antibodies, proteins and peptides are used. When targeting the drug to the kidney, the main targets are the glomerular mesangial cells and proximal tubular cells [87,88].

Macromolecular carriers

To target the drug to the kidneys, macromolecular carriers are reported to deliver the drug effectively. Generally, biologically active low-molecular-weight proteins (LMWP) having smaller molecular weights (<30,000 Da) are used. LMWPs include immune proteins (light chain immunoglobulin), enzymes (lysozymes) and peptide hormones (insulin). These get filtered at the glomerulus and reabsorbed in the renal tubules. The presence of LMWP overrules the kinetics of the drug [87].

The linking of LMWP with drugs to form the conjugate can be done by various methods involving the formation of amide (lysine amino group, oligopeptides), ester ((poly)-α-hydroxy acids), disulfide and peptide bonds, which can be then cleaved to release the drug by either chemical or enzymatic hydrolysis. While synthesizing the LMWP–drug conjugate, careful control of the reaction is required as the LMWPs contain numerous active groups and are prone to self-aggregation [88].

Lysozymes

Lysozyme, an endogenous protein with a molecular weight of 14 kDa, is the most explored LMWP. When used as a carrier, lysozyme offers many advantages, such as selective renal uptake, cell-selective drug release, easy conjugate formation and biocompatibility [87,88]. In the study conducted by Shibata *et al.*, it was observed that on the administration of lysozyme in the diabetic nephropathic kidney, the thickening of the GBM was reduced [89]. When the ACE inhibitor captopril was attached via a disulfide bond to the lysozyme using a spacer, a sixfold increase in captopril concentration was observed [88].

Hass *et al.* developed and studied the *in vivo* renal delivery of a naproxen–lysozyme conjugate for kidney-targeted drug delivery, and showed rapid accumulation of the conjugate in the kidneys. After accumulation in the kidney, an active form of naproxen, naproxane-lysine, was formed, which inhibits cyclooxygenase [90]. In the investigations performed by Zheng *et al.*, baicalin, a bioactive component of *Scutellaria baicalensis*, was linked with lysozyme to assess its anti-inflammatory and renoprotective activity. This conjugate showed renal fibrosis and inflammation inhibition via NF-κB, TGF-β1/Smad3 and IGF-1/p38 MAPK signaling pathways [91].

Polymers

In recent years polymers have played an essential role in drug delivery because their biocompatibility helps in controlled and targeted delivery. Molecular weight, charge, functional groups, distribution and accumulation of polymer in the body are critical parameters that directly influence drug efficiency and targeting capacity. There are few polymers reported as carriers for the delivery of drug at the renal site. The clearance of the polymers generally decreases with increasing molecular weight of the polymer. The drug–polymer complex presents various benefits like better specificity, reduced adverse effects and an increase in efficacy [92].

Chitosan

Chitosan is a natural copolymer comprising glucosamine and N-acetyl-glucosamine, which are derived from chitin. Chitosan has excellent properties of biocompatibility, biodegradability and low toxicity. As a biomaterial, it has been extensively studied for its potential to serve as an antidiabetic supplement and as a platform for delivery of insulin and imaging of islet cells [93]. To develop a renal-targeted drug delivery system, low-molecular-weight chitosan (LMWC) is widely used. For the assessment of renal targeting using polymers as a carrier, polymers with variable degrees of acetylation and molecular weight are studied. It has been observed that the renal clearance of LMWC–drug conjugates compared with lysozyme–drug conjugates is rapid and hence has a safe pharmacokinetic profile [87,94].

In the investigation performed by Yuan *et al.* to identify the renal targeting of chitosan, prednisolone was conjugated with 50% N-acetylated LMWC. This conjugate exhibited selective accumulation in the kidney, which helped in delivering the prednisolone to the kidneys. Prednisolone was conjugated with 19-kDa LMWC, and a 13-fold increase in the concentration was observed [95–97].

Poly(vinylpyrrolidone-co-dimethyl maleic anhydride)

Tsutsumi *et al.* reported the application of a poly(vinylpyrrolidone-co-dimethyl maleic anhydride) (Poly(VP-co-DMMAn)) copolymer for renal drug targeting, first synthesizing the poly(VP-co-DMMAn) copolymer using vinylpyrrolidone and dimethyl maleic anhydride as a monomer. The co-polymer was rapidly eliminated from the blood but accumulated in the renal proximal tubular cells of the kidney [98]. The poly(VP-co-DMMAn) copolymer was conjugated with superoxide dismutase for the treatment of renal disease. Kuo *et al.* observed the effect of extracellular superoxide dismutase in diabetic nephropathy and found that it inhibits ROS generation and the ERK signaling pathway [99]. The result of the *in vivo* study using male BALB/c mice revealed that after 24 h of intravenous administration, nearly 80% of the 10-kDa poly(VP-co-DMMAn) was found to be selectively accumulated in the kidneys [98].

Poly(vinylpyrrolidone-co-dimethyl maleic acid) & anionic polyvinylpyrrolidone derivatives

It is reported that poly(vinylpyrrolidone-co-dimethyl maleic acid) (PVD) has a potential for high accumulation in the kidney and can be conjugated with superoxide dismutase for the treatment of renal disease. The result of an *in vivo* study using male BALB/c mice revealed that the PVD's molecular weight and charge influence the distribution of the polymer. The *in vivo* result indicated that polymer with a molecular weight of 6–8 kDa shows high renal accumulation, and about 80% is selectively distributed in the kidneys after intravenous administration. The higher the negative charge of PVD, the more time is required for its elimination from the kidney [100].

Tsutsumi *et al.* also evaluated various anionic polyvinylpyrrolidone (PVP) derivatives for renal drug targeting. The *in vivo* data showed that carboxylated PVP was efficiently taken up by the proximal tubular epithelial cells compared with sulfonated PVP. This study exhibits that carboxylated PVP has high renal targeting properties [101].

Poly-L-glutamic acid

Chung *et al.* demonstrated poly-L-glutamic acid (PG) as a selective renal polymeric drug carrier for renal protecting drugs. PG is a nonimmunogenic, biodegradable and biocompatible polymer with excellent drug loading capacity [102,103]. In this study, 17-kDa and 41-kDa PG polymers were evaluated for renal targeting in a male Sprague Dawley diabetic rat model induced by streptozotocin. The authors assessed the accumulation of PG in both the streptozotocin-induced model and normal rats by labeling PG with ^{3}H-deoxycytidine. After 24 h of administration, the 17-kDa PG and 41-kDa PG, both labeled with ^{3}H-deoxycytidine, showed sevenfold and 15-fold higher accumulation in the kidney, respectively, compared with free radiolabel [104]. The renal filtration threshold is about 30 kDa. The polymer of molecular weight above the renal filtration threshold (i.e., the 41-kDa PG) showed a better accumulation profile than the 17-kDa PG.

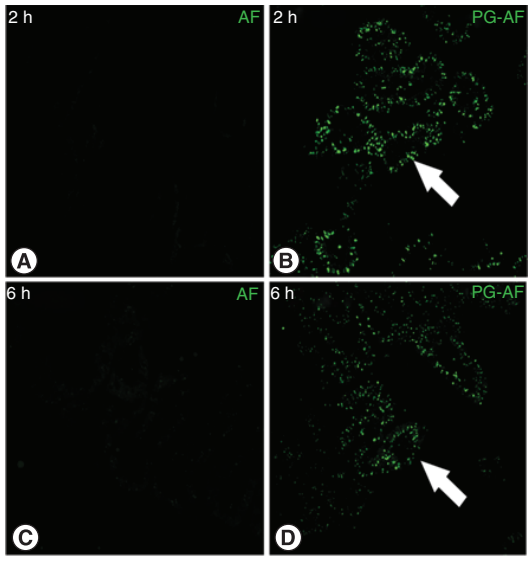

Figure 3. Fluorescence imaging of normal rat renal tissues. **(A)** 2 h after free AF treatment. **(B)** 2 h after 41-kDa PG-AF. **(C)** 6 h after free AF treatment. **(D)** 6 h after 41-kDa PG-AF. The green fluorescence depicts the AF compound. No indication of fluorescence was seen in free AF-treated animals.
AF: 5-(aminoacetamido) fluorescein.
Modified with permission from [104] © Dove Medical Press (2017).

PG was conjugated with 4-(2-aminoethyl) benzene sulfonyl fluoride hydrochloride (AEBSF) to form PG-AEBSF in a study comparing the antioxidative stress activity of PG-AEBSF with that of free AEBSF [104]. The *in vitro* data showed that PG-AEBSF had antioxidative stress activity the same as that of the free AEBSF. In another study, PG of molecular weight 41 kDa was labeled with 5-(aminoacetamido) fluorescein (fluoresceinyl glycine amide) to form PG-AF, and the selective uptake of PG-AF by the epithelial cells of the kidney was investigated. Accumulation of free AF and 41-kDa PG-AF in epithelial cells of the proximal tubule was checked at 2 and 6 h after the intravenous administration. Fluorescence was seen in PG-AF-treated animals, but not in free AF-treated animals, as depicted in Figure 3 [104]. The renal retention of polymer with a molecular weight lower than the renal filtration threshold involves active reuptake of polymers by the proximal tubules, a post-glomeruli process, whereas the renal retention of higher molecular-weight polymer generally involves extravasation from the renal vasculature and deposition in the parenchymal tissues [101].

Peptides & antibody ligands

Peptides are polymers of amino acids, which are simple to synthesize and easily degradable in the body. Specific interactions between peptides and receptors can be exploited for targeted drug delivery. Advances in phage display technologies have increased the number of peptides for site-specific delivery to the kidney. C peptide is a short sequence of 31 amino acids that connects the A and B chains of the proinsulin molecule. Apart from the structural connectivity role of insulin, C peptide also plays an essential role in metabolic functions. It binds via the pertussis toxin-sensitive G-protein dependent receptor, decreases oxidative stress and inflammatory response and controls various intracellular signaling pathways in the kidney [105,106].

In previous studies, polyethyleneimine was considered to have high transfection ability but also high cytotoxicity [107]. To overcome polyethyleneimine's cytotoxicity, the poly(ester amine) (PEA) was developed to have high transfection and low cytotoxicity. Cho *et al.* conjugated PEA with the kidney-targeting peptide LTCQVGRVH, and found that the PEA–peptide conjugate exhibited specific delivery of the *HGF* gene to the obstructed kidney in unilateral ureteral obstruction model rats [108]. This PEA–peptide conjugate shows lower cytotoxicity and can be used as an effective nonviral carrier for gene delivery.

Yuan *et al.* reported the targeting ability of the peptide fragment PF-$A_{299–585}$ by conjugating the peptide with triptolide. $A_{299–585}$ is the peptide fragment of human serum albumin, and triptolide has a potent anti-inflammatory action, as per Chinese traditional medicine. This peptide conjugate accumulates in the inner lining of renal tubules by endocytosis. The *in vitro* data exhibited that the conjugate was less cytotoxic but had an anti-inflammatory action the same as that of the plain triptolide drug. The *in vivo* data confirmed a rapid accumulation of the conjugate in the kidney and showed that it prevented the worsening of the condition in the rat model. The results prove that the targeting potential of the conjugate can be used for curing DN. Figure 4A shows the biodistribution of the triptolide–peptide conjugate in the kidneys and other organs. Figure 4B shows that the fluorescence intensity was

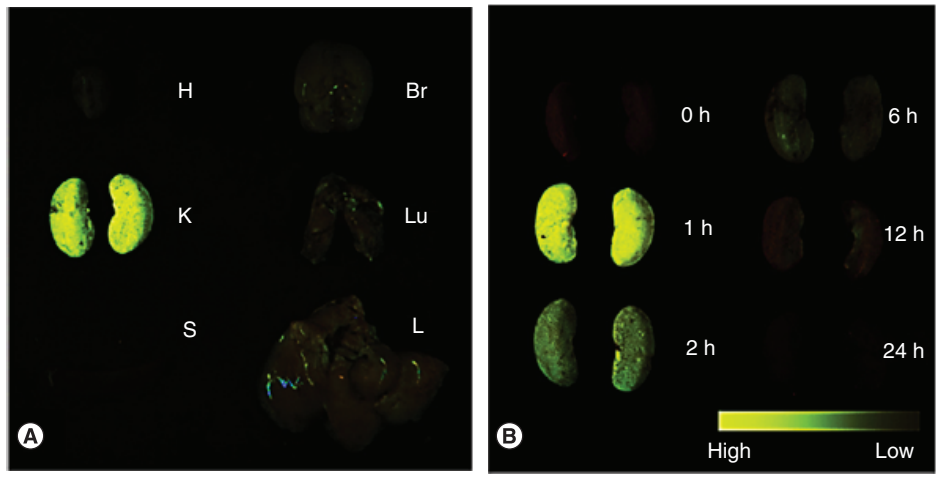

Figure 4. Histology of tissues harvested from mice following intravenous administration of triptolide–peptide conjugate. (A) Tissues from the heart (H), kidneys (K), spleen (S), brain (Br), lungs (Lu) and liver (L) harvested at 1 h after administration of conjugate. **(B)** Kidneys imaged at different time intervals after administration show sustained renal retention of the conjugate.
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highest after 1 h of administration of triptolide–peptide conjugate and decreased afterward, demonstrating that the maximum triptolide–peptide conjugate reaches the kidney in 1 h and the uptake decreases after that [109].

Vadhatpour *et al.* investigated the effects of different glycine-, alanine-, leucine-, proline- and histidine-based peptides on kidney protective effect in streptozotocin-induced DM male BALB/c mice. Carnosine dipeptides (comprising alanine and histidine) and hannaneh dipeptides (comprising leucine and glycine) showed promising results by hematoxylin and eosin staining and using various enzymatic biochemical assays [110].

In previous studies, pigment epithelium-derived factor (PEDF) was considered to have high neuroprotective and anti-inflammatory activities [111]. In DN, the level of PEDF decreases in the kidney. PEDF is a bulky protein with an active peptide fragment P78-PEDF, comprising 44 amino acids. Awad *et al.* showed a protective action of P78-PEDF peptide in streptozotocin-induced DM DBA/2J mice [112]. A further study proved that delayed treatment with this peptide inhibits DN progression [113].

In an investigation by Wischnjow *et al.*, the uptake flow of LMWP was explained. They synthesized a peptide, $(KKEEE)_3K$, as a drug carrier and studied its uptake in the renal tubules of C57BL/6J mice. The immunohistochemical staining and pharmacokinetic study results confirmed the accumulation of the peptide in the renal tubule cells. It was also observed that the peptide was stable for at least 24 h in human serum without any degradation [114].

Active targeting is an efficient approach for targeted delivery employing a homing moiety, which is part of an adaptive system like antibodies. An antibody is a complex protein-based molecule generated by the body's immune system in response to an antigen. Antibodies with a molecular weight of >150 kDa cannot pass through the glomerular filtration barrier; thus antibodies are problematic as targeting carriers for the proximal tubular cells. Antibody fragments with molecular weight <50 kDa seem to be a promising targeting moiety to target kidney cells like glomerular endothelial cells, proximal tubule cells and basement membrane [115]. Hultman *et al.* developed immune-targeted iron oxide nanoparticles for drug delivery and diagnostics specifically for the kidney and kidney tissues [116], while Yasuhiko *et al.* designed anti-CD11b surface-modified silica nanoparticles for site specificity to renal damage. Renal inflammation imaging was observed after intravenous administration of the fluorescent-labeled anti-CD11b surface-modified silica nanoparticles in unilateral ureteral obstruction model mice [117].

Prodrug approach

A prodrug is the inactive form of a drug, which is activated by metabolism or by another enzyme's action. It has been observed that sugar-, folate- and amino acid-modified prodrugs could be exploited for renal targeting [87]. The renal tubules are lined by specific receptors, like folate receptors and particular enzymes, and these receptors are generally involved in the uptake of the prodrug. Wang *et al.* studied the uptake of a diethylenetriamine pentaacetic acid–folate conjugate. Compared with other organs, they found that a large amount of folic acid conjugates

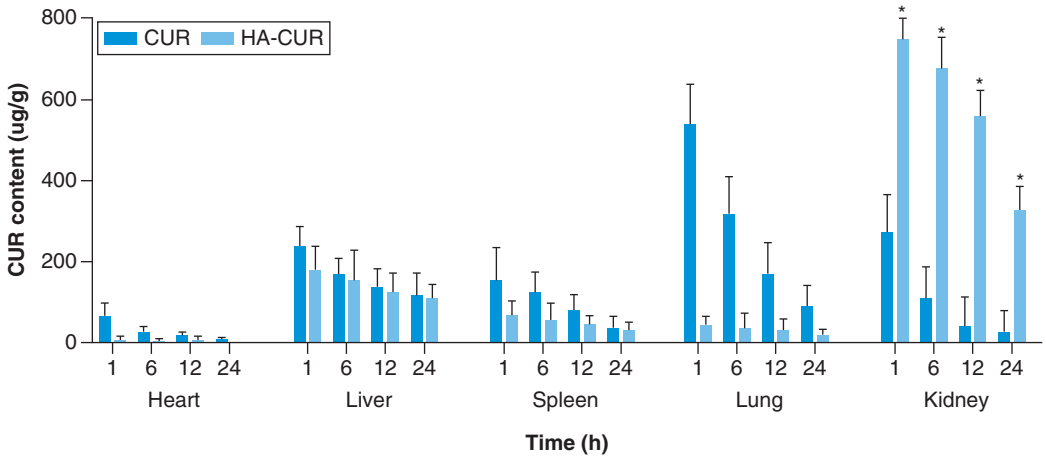

Figure 5. Distribution of free curcumin and hyaluronic acid–curcumin prodrug in different organs of murine models.
Time points at 1, 6, 12 and 24 h after intravenous administration at a dose of 10 mg/kg curcumin, respectively.
CUR: Free curcumin; HA–CUR: Hyaluronic acid–curcumin.
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were distributed in the kidney [118]. Li *et al.* observed the effect of modifying prednisolone on its uptake in HK-2 cells. It was found that the modified glycated prednisolone derivative exhibited a more than fourfold increase in the uptake of sugar prodrug of prednisolone compared with unmodified prednisolone [119].

Golshayan *et al.* designed a peptidase-targeted prodrug using tetrapeptide SS-31. Angiotensin receptors and aminopeptidase A play a vital role in RAAS, regulating the kidneys' functions. The SS-31 tetrapeptide prodrug decreased the expression of AT_1R mRNA and upregulated the expression of AT_2R. SS-31 tetrapeptide-based prodrugs act as substrates for aminopeptidase A and protect the kidney from damage [120]. Du *et al.* designed a hyaluronic acid–curcumin prodrug in which hyaluronic acid and curcumin were conjugated via an ester linkage. Hyaluronic acid not only increased the solubility of the curcumin, but it also helped in targeting the CD44 receptors, which are upregulated on kidney tubular cells in cases of kidney injury. Biodistribution studies demonstrated that the hyaluronic acid–curcumin prodrug was highly accumulated in the kidney compared with the other organs, as depicted in Figure 5 [121].

Nanoformulation-based approaches for glomerulus targeting

In the nephritic state, nanoparticles have a high inclination for deposition into the glomerulus due to enhanced vascular permeability and localized inflammation. By exploiting the presence of various targeting windows within the diseased nephron (e.g., size cutoff, presence of charge-bearing constituents and availability of specific receptors), functional nanoparticles for targeted therapeutic and diagnostic applications can be engineered. The various approaches for targeting nephrons are shown in Figure 6 [122].

Podocytes

Podocytes, also known as glomerular epithelial cells, are specialized terminally differentiated cells of the kidney glomerulus, having a complex cellular architecture that involves the cell body, mutual connection and highly branched foot processes (FPs). The FPs diverge from the cell body and form a remarkably selective filtering apparatus by draping the GBM [124]. Through these 'filtration slits', which have a size cutoff of approximately 10 nm, podocytes prevent the entry of plasma proteins into the urinary filtrate, only allowing small solutes. The proper functioning of podocytes is essential for efficient glomerular filtration and renal function [125].

DN is associated with a decrease in podocyte number, effacement of podocyte FPs and loss of filtration slit proteins (nephrin and podocin), which leads to leakage of albumin. Being postmitotic cells, podocytes have a limited proliferation capacity, and injury to podocytes and the resultant albuminuria (a sequenced process that involves podocyte hypertrophy, podocyte epithelial–mesenchymal transition, podocyte detachment and podocyte apoptosis) have been identified as pivotal events in DN [126,127]. Much research has been carried out to identify

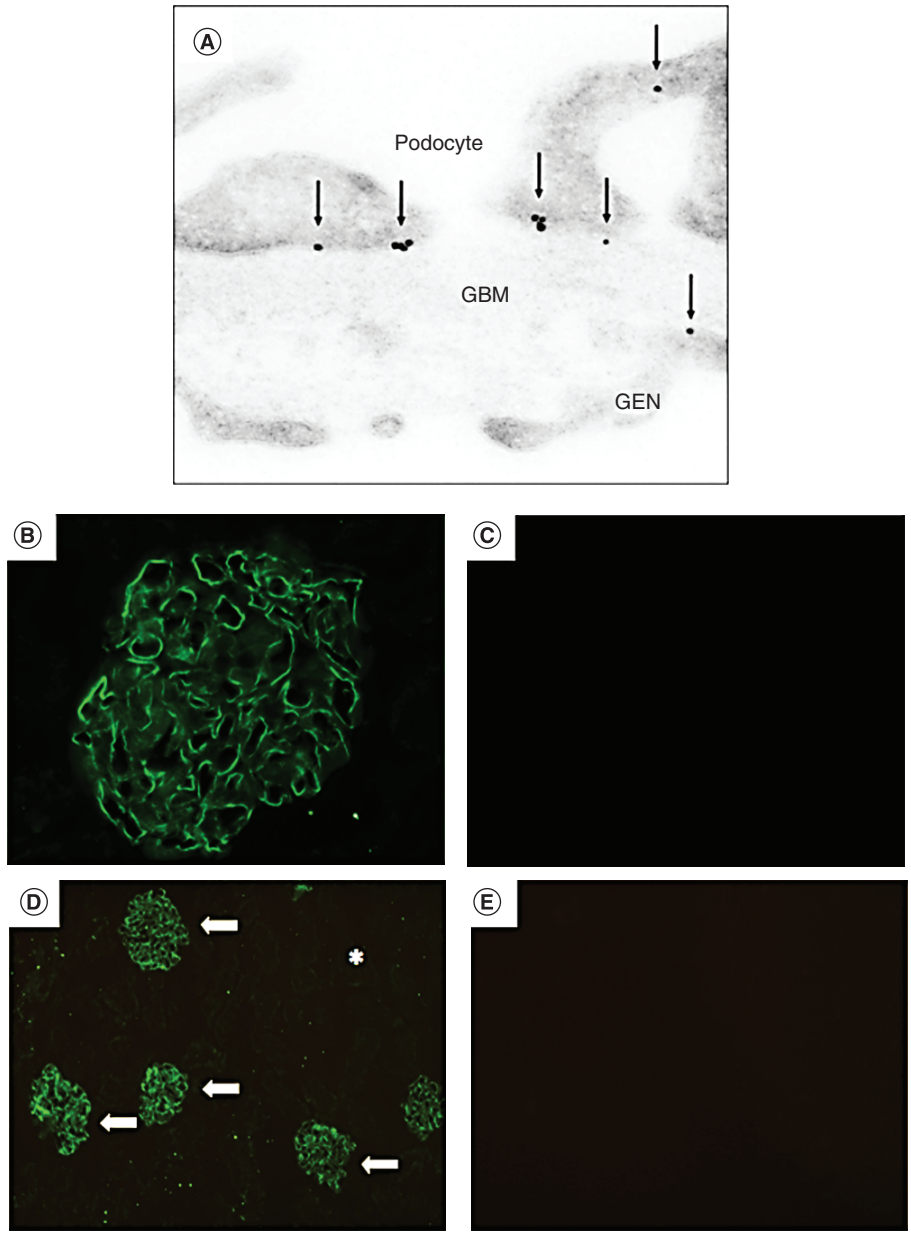

Figure 6. Affinity of the antipodocyte antibody. (A) The antibody binds to the apical, basal and slit diaphragm regions of the podocyte. **(B & D)** A small number of immunogold particles were present in the glomerular basement membrane (GBM) and endothelial cells (GEN). The arrows indicate examples of immunogold particles. Sheep anti-mouse podocyte antibody staining was detected only in the glomerulus and was absent in the tubular compartment **(D)**. **(C & E)** Control animals injected with normal sheep IgG showed no staining.
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novel targeted therapies that can serve efficiently to deal with DN by halting or preventing the complications associated with podocyte injury.

To circumvent the systemic side effects accompanying the use of glucocorticoid therapy in nephrotic syndrome (an outcome of DN), Wu *et al.* developed monomolecular nanoconjugates by exploiting an acid-sensitive amide bond to link methylprednisolone (as the drug) to albumin (as a carrier having the targeting property). The prepared nanoparticles exhibited uniform and monodispersed size distribution, possessing an average diameter of approximately 10 nm which would be enough to pass through renal glomerular filtration in pathological conditions. Albumin facilitates localized delivery into cultured human podocytes because it is a specific ligand of the neonatal Fc

receptor (FcRn), which is highly expressed in podocytes. The nanoconjugates showed extensive receptor-mediated, dose-dependent uptake in human podocytes. The acid-sensitive linkage is cleaved by cellular lysosomes, allowing drug release from the nanoconjugate after cellular uptake [128]. With a similar objective of delivering glucocorticoid locally to repair injured podocytes, Colombo *et al.* developed a library of biocompatible polymeric nanoparticles having tunable sizes and surface properties. Neutral, positively charged and negatively charged poly(ε-caprolactone)-based nanoparticles were prepared by copolymerization with polymerizable methacryloyl surfactants, producing particle sizes in the range of 30–120 nm. The cytotoxicity and uptake of the nanoparticles depended on their size and surface charge. The sustained therapeutic effects of encapsulated drugs were demonstrated on podocyte cultures, with the damaged podocytes being effectively repaired by the controlled delivery of dexamethasone [129].

In another study, Akilesh *et al.* observed that podocyte FcRns play a role in the transport of IgG and albumin, as mice deficient for FcRn show characteristic accumulation of IgG in the GBM via statin-dependent endocytosis [130]. Based on this, Hauser *et al.* generated an anti-mouse podocyte antibody that binds to rat and mouse podocytes *in vivo* for delivery of therapeutic siRNA (Figure 6). Utilizing the available sulfhydryl group, monovalent IgGs were linked with neutravidin. Further, protamine (a polycationic nuclear protein and universal adaptor for anionic siRNA) was linked to the neutravidin via biotin. Injection of the antibody-based delivery system (coupled with either nephrin siRNA or TRPC6 siRNA) via the tail vein into normal rats substantially reduced the protein levels of nephrin or TRPC6 respectively. The effect was target-specific, as other podocyte-specific genes remained unchanged. This study showed for the first time that siRNA can be delivered efficiently and specifically to podocytes *in vivo* using an antibody-based delivery system [123].

Mesangial cells

Mesangial cells are irregularly shaped contractile cells that constitute the central stalk of the glomerulus. The term 'mesangium' refers to mesangial cells along with the mesangial matrix. In association with endothelial cells and podocytes, the mesangial cells create a functional glomerular unit. Their contractile properties modulate the intraglomerular capillary flow and the glomerular filtration surface area [131]. Any direct or indirect injury to mesangial cells plays a crucial role in developing ESRD, leading to cell proliferation, mesangial expansion, hypertrophy and extracellular matrix accumulation [132]. As mesangial cells are critical in the commencement and progression of DN, the prospect of selectively modulating the mesangial cell by external administration of a therapeutic drug makes it attractive as a novel therapeutic approach [133].

To develop a platform for targeted therapeutic siRNA delivery to the glomerular mesangium in humans, Zuckerman *et al.* prepared and evaluated siRNA-loaded nanoparticles using polycationic cyclodextrin (siRNA/CDP-NPs) [134]. As mannose receptors are selectively expressed in mesangial cells, using the sugar mannose as the surface targeting ligand facilitated prolonged glomerular localization of siRNA/CDP-NPs. Epifluorescence experiments qualitatively verified the substantial mesangial cell uptake for targeted nanoparticles. Gene silencing in the mesangium following intravenous administration was evaluated by measuring the extent of downregulation of enhanced green fluorescent protein production in *EGFP*-expressing transgenic mice and mesangial cell lines. This study showed that in both mouse and human mesangial cells, siRNA/CDP-NPs were rapidly internalized rather than free siRNA *in vitro.* Surface targeting ligands can enhance the uptake of the siRNA/CDP-NPs by mesangial cells.

Because of the unique anatomy within the renal corpuscle, nanoparticles show a size-dependent accumulation. Particles with sizes in the range of 80–150 nm tend to accumulate passively after passing through the pores of fenestrated endothelia between mesangial cells and the extracellular matrix. This size-dependent accumulation provides a window to develop new therapeutic interventions. Based on this, Choi *et al.* developed gold-based nanoparticles with surface PEGylation (using gold-thiol chemistry) as a nano platform for therapeutic and diagnostic applications [135]. PEGylated gold nanoparticles with a size of 80 nm and a zeta potential of -10 mV showed significant uptake by mesangial cells. Using the same principle, Li *et al.* developed poly(D,L-lactic-co-glycolic acid) (PLGA)-derived nanoparticles for the delivery of dexamethasone acetate into glomerular mesangium [136]. The nanoparticles displayed rapid internalization, and the selective accumulation of PLGA–PEG nanoparticles of size 90 nm was confirmed by confocal microscopy.

Glomerular basement membrane

The GBM is a trilayered extracellular matrix that originates from the union of the respective basement membranes of podocytes and glomerular endothelial cells [136]. The presence of over 140 distinct proteins (confirmed via proteomic

analysis of purified human GBM) makes the composition of GBM quite complex. Among these proteins, collagens (types I, IV, VI and XVIII subunits), laminins (α5, β2 and γ1), nidogen-1, heparan sulfate proteoglycans (agrin and perlecan) and TINAGL1 are most abundant [137]. Collagen IV acts as the chief structural protein and forms the characteristic cross-linked network, having a fibrous lattice with heterogenous pores that facilitate the anchoring of other structural components. A crucial component of the glomerular filtration barrier, the GBM acts as an intermediate sieve and a supporting framework that allows the proper functioning of glomerular endothelium and podocytes [138].

Any structural damage can lead to abnormalities and loss of the GBM's size selectivity, leading to albumin leakage and proteinuria. The podocyte–GBM interface is disturbed in the diabetic milieu, with thickening of the GBM being an early marker of DN progression. From the perspective of drug delivery, positively charged nanoparticles are quickly accumulated within the GBM. One possible reason behind this is the basement membrane's anionic nature due to a large amount of heparan sulfate-containing glycocalyx [139].

Based on this, Zuckerman *et al.* prepared positive charge-bearing cyclodextrin-based siRNA nanoparticles (size: 80 nm; ζ potential: +10 mV), which displayed specific interaction with anionic GBM [140]. Similarly, in a previous study, Bennett *et al.* utilized the electrostatic interaction between cationic nanoparticles and anionic GBM to deliver iron oxide nanoparticles as a contrast agent for detecting the basement membrane's breakdown in disease [141].

Proximal tubule

Within the nephron, the proximal tubule is actively involved in the reabsorption of glomerular filtrate, including essential ions, water and small molecules. Furthermore, practically 100% of the filtered amino acids, glucose and small peptides are reabsorbed in this region. The renal proximal tubular epithelial cell (PTEC) plays a vital part in the pathogenesis of DN [142]. PTECs orchestrate kidney injury in DN's progression through the action of several vital substrates of DM that trigger key proinflammatory and fibrogenic signaling pathways. The accumulation of fibronectin, AGEs and various inflammatory mediators is responsible for fibrosis in renal tubules [19]. Even though DN was conventionally considered a glomerular disease, at present it is commonly recognized that the rate of deterioration of function correlates best with the degree of renal tubulointerstitial fibrosis, which can be characterized as a progressive detrimental connective tissue deposition on the kidney parenchyma, leading inevitably to renal function deterioration [143]. Advanced tubulointerstitial fibrosis is a common pathway for all kidney diseases leading to chronic renal failure, and microalbuminuria is known to activate the PTECs to induce tubulointerstitial inflammation [144].

For targeting PTECs, Gao *et al.* prepared siRNA-loaded chitosan nanoparticles as a proof of principle for silencing specific genes expressed in PTECs during kidney diseases such as DN [145]. siRNA therapy attracts much attention due to its efficiency and specificity, but its therapeutic use is still limited due to insufficient circulation time and biodistribution, mainly dependent on its encapsulation into the carrier entity. Taking advantage of PTECs' tendency to take up low-molecular-weight chitosan via the scavenging receptor megalin, the chitosan polyplex nanoparticles showed specific localization in PTECs, confirmed by *in vivo* fluorescence bioimaging and immunohistological analysis. Moreover, the nanoparticles displayed uptake via the megalin-dependent endocytosis pathway and delivered the siRNA cargo, and successfully silenced *AQP1* in the PTECs. Williams *et al.* developed a kidney-targeted mesoscale nanoparticle designed from a diblock copolymer comprised of PLGA–PEG [146]. The nanoparticles were localized in the kidneys (specifically in the proximal tubules via peritubular capillaries) with more than 26-fold efficiency over other organs. The nanoparticles displayed excellent biocompatibility and safety (as expected given the FDA-approved polymers that constituted the nanoparticles).

In an exploratory study, Williams *et al.* proposed the use of mesoscale nanoparticles (composed of PLGA−PEG), synthesized by a nanoprecipitation method and having an approximate size of 400 nm, for selective localization in renal proximal tubules. Use of a nonopsonizing material like PEG allows evasion from mononuclear phagocyte system-mediated phagocytosis (responsible for off-target accumulation of nanoparticles in the liver and spleen). The results of *in vivo* biodistribution studies suggest a seven-times higher localization in the kidney when compared with other organs (Figure 7). The authors proposed that both the cationic and the anionic mesoscale nanoparticles undergo endocytosis by endothelial cells of the peritubular capillaries because of the pressure drop in the nephron and the large absorptive pressure of the capillaries. By applying such a targeting strategy, mesoscale nanoparticles could be used to treat diseases that affect the proximal tubules of the kidneys and to study renal blood flow [147].

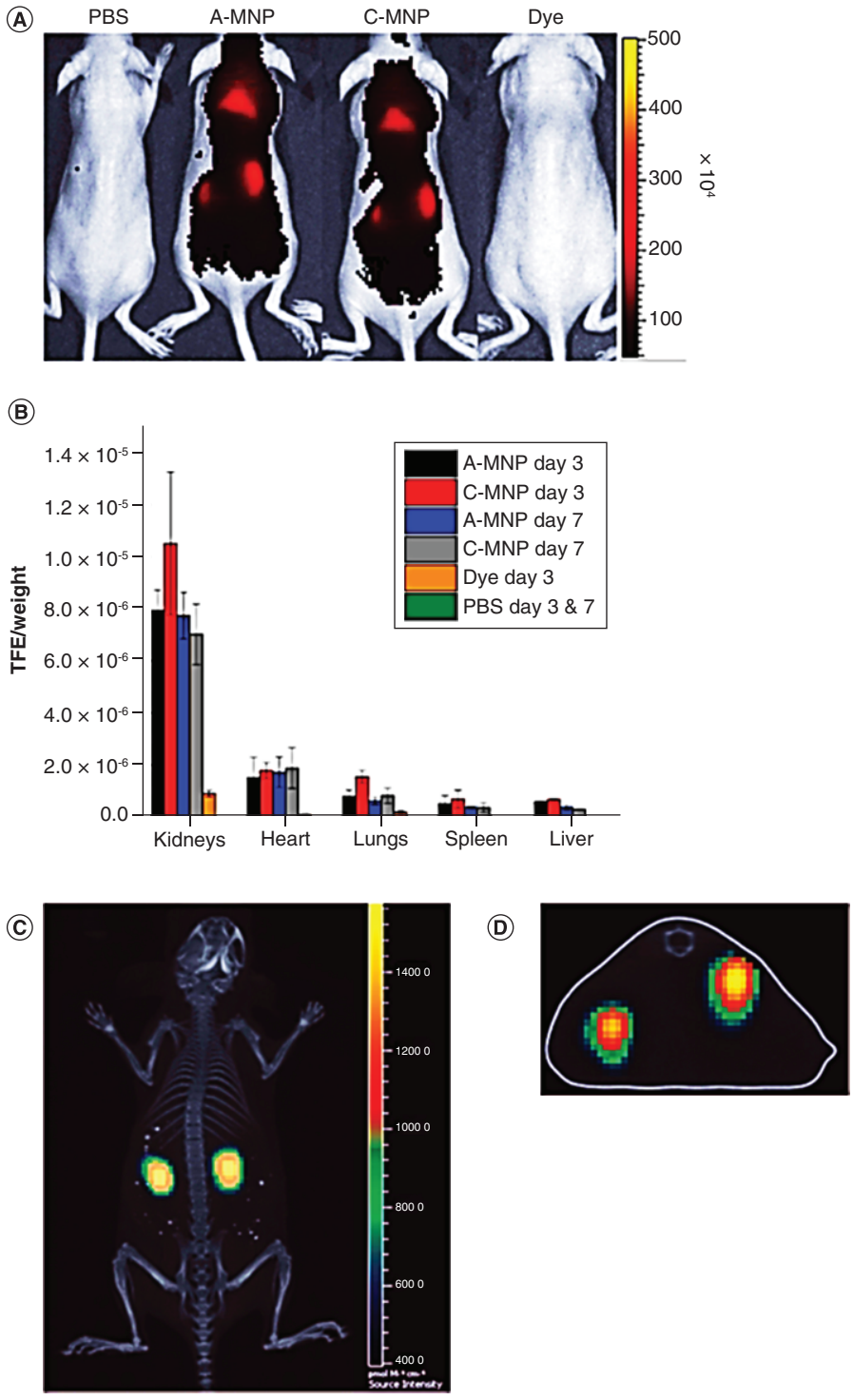

Figure 7. ***In vivo*** **biodistribution of mesoscale nanoparticles. (A)** Dorsal image of mice treated with PBS, 50 mg/kg A-MNPs, 50 mg/kg C-MNPs and an equal molar weight of free dye on the day they were sacrificed. **(B)** *Ex vivo* organ fluorescence from mice injected with MNPs, dye or PBS normalized by total organ weight (mean ± SD). **(C)** Fluorescence plus CT overlay focused on the kidneys of a mouse treated with A-MNPs, showing localization and relatively homogeneous distribution throughout the kidneys. **(D)** Fluorescence plus CT transaxial section of a mouse treated with A-MNPs, showing bright fluorescence throughout the kidneys.
A-MNP: Anionic mesoscale nanoparticles; C-MNP: Cationic mesoscale nanoparticle; CT: Computed tomography; MNP: Mesoscale nanoparticle; PBS: Phosphate-buffered saline; SD: Standard deviation; TFE: Total fluorescence efficiency.
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Conclusion

DN has become a global impediment associated with Type 1 and Type 2 DM. As DN is a complex condition involving various mechanisms, understanding the molecular pathways (along with the transcription factors and mediators like NF-κB and TGF-β that play a vital role in the progression of DN) and the molecular targets for DN is essential for targeted delivery at a specific site. To treat this condition, targeted drug delivery at a localized area is a promising strategy. Renal targeted drug delivery is of significant interest among many research groups for the treatment of DN. Polymers, peptides and various macromolecules are explored as carriers for targeted drug delivery to the kidney. The use of nanoformulations is an emerging approach that can deliver the drug to the targeted site efficiently. Various nanoformulation approaches targeting different sites are being explored.

In the case of polymeric carriers, the renal filtration threshold plays an important role. The renal retention of polymer with a molecular weight lower than the renal filtration threshold involves the post-glomeruli process. In contrast, the renal retention of polymers with higher molecular weights generally involves extravasation from the renal vasculature and deposition in the parenchymal tissues. Various carriers have also been installed with ligands for efficient and site-specific delivery of diagnostic or therapeutic agents to the kidney cells.

Future perspective

Designing an optimal platform for drug delivery within the kidneys for the treatment of DN is an extensively complex challenge faced by formulation scientists, considering the ability of the kidney to excrete drugs out of the body. The domain of nanomedicines is bound to capture a significant market share in the near future, as evident from the abundant nanoparticles already available on the market and the numerous others undergoing clinical trials for approval. In relation to DN, nanomedicine-based approaches with tailored biophysical characteristics allow improved interaction with renal cells to achieve enhanced retention as well as improving cellular uptake within the GBM epithelial cells, podocytes, mesangial cells and proximal tubule cells. As discussed here, important pioneering studies have commenced, but focused pharmacokinetic and pharmacodynamic studies in pathological conditions that mimic characteristic albuminuria, along with the altered GFR seen in DN, need to be done to evaluate the applicability of nanomedicine. Information pertaining to the structure and function of the kidney, combined with new insights into the pathogenesis of DN, serve as powerful tools for optimization of drug delivery systems for the clinical treatment and research of DN. It is time that the nanotechnological research teams and nephrology experts collaborate to develop encouraging nanomedicine tactics for the selective targeting and treatment of DN.

Executive summary

Diabetic nephropathy: a long-term kidney disease

- Diabetic nephropathy (DN) is the foremost cause of end-stage renal disease.
- It occurs as a consequence of the interaction between metabolic and hemodynamic factors.
- With a rise in the number of diabetic patients, the occurrence of DN is expected to surge up to pandemic proportions.
- DN's vasoactive pathway includes alterations in the renin–angiotensin–aldosterone system, hypertrophy of the mesangium and tubular epithelial cells, and endothelin receptor-mediated DN progression.
- Metabolic pathways include the generation of reactive oxygen species, the activation of PKC, advanced glycation end products and polyol pathways, and the release of transcription factors like NF-κB.

Molecular biomechanics of DN

- DN can be diagnosed using various protein, podocyte and tubulointerstitial biomarkers.
- DN treatment demands high renal concentrations of drugs, which mandates the development of targeted drug delivery systems.
- Current available DN therapeutic interventions emphasize blood pressure, glycemia and lipid control, while ignoring DN's progression mechanism at a molecular level.

Emerging strategies for DN targeting

- Targeted nanoparticles for diagnosis and therapy can be engineered according to pathophysiological conditions (e.g., size cutoff, presence of charge-bearing constituents and receptor overexpression).
- Lysozymes, peptides, chitosan, poly(vinylpyrrolidone-co-dimethyl maleic anhydride), poly-L-glutamic acid and polyvinylpyrrolidone derivatives are widely used macromolecular carriers.
- Considering and connecting the molecular insights involved in DN helps understanding of the initiation and progression pattern.
- Novel strategies with reported applications in developing a nanomedicine-based platform for DN-targeted drug delivery to improve drug efficacy and safety are a promising strategy for the future treatment of DN.

Financial & competing interests disclosure

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